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The Commissioner of Patents

14 December 2005

Madam

IN THE MATTER OF International Patent Application No. PCT/AU2004/001577
in the name of COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION
entitled METHOD FOR MICROBE AND ENZYME DISCOVERY
Our Ref: JSB:SP:FP20705

We refer to the Written Opinion dated 17 November 2005.

As this response is filed at least 3 months before the Final Date for the establishment of the international preliminary examination report (IPER), we look forward to receiving further comments from the Examiner if she believes that any matters remain outstanding that prevent the issuance of a clear IPER, so that a further response can be filed if required.

We enclose a revised page 63, containing revised claim 1, to be substituted for page 63 presently on file. The amendments to claim 1 further clarify the scope of the present invention. In particular, we note that the metabolism indicators mentioned in claim 1 are specified to be "terminal electron acceptors", these molecules being ones that are involved directly in the aerobic and anaerobic respiration of microorganisms. This amendment is based on the specification at page 16 line 14, and elsewhere. This takes us away from the issue raised by the Examiner with respect to previous claim 1 not precluding the measurement of various products of metabolism of the substrate, which were not intended to be encompassed. We assert that it was not commonly known that assessment of the change in the level of a terminal electron acceptor (such as the rate of oxygen consumption), which comes from measuring the level of the terminal electron acceptor over time, can provide a direct correlation to microbial test substrate metabolism – and thus enrichment. This choice of measurement technique gives the advantages of broad applicability (i.e. no matter the product, the enrichment can be monitored). This is of particular interest in the area of identifying microorganisms for metabolising test substrates – where the range of substrates for which you wish to find a microbial "enemy" can vary widely. We appreciate that wide applicability is not determinative of novelty, but it must be considered to be a factor when considering inventive step – the fact that the prior art methods cannot be applied so broadly as to work irrespective of the test substrate, must be a factor indicating that there is an inventive step.

Revised claim 1 also refers to the output being based on the *change* in level of the metabolism indicator (i.e. terminal electron acceptor) – which is different to those prior art processes that merely measure dissolved oxygen levels. The change in level of metabolism indicator in turn is relied upon to (quantitatively) correlate detection of the metabolism indicator to actual enrichment. Basis for this is found at page 16 line 21, and the examples where the calculations show that the change in level of metabolism indicator (for example, oxygen update *rate*) is utilised.

Revised claim 1 also specifies that the signal is produced from a probe that takes a reading in the vessel. This clarification serves to show that this is distinct from prior art process where samples are taken out of the vessel ("off-line") to be tested. Even when this is done in the prior art, the data collected is never shown to be used to create an output (especially electronically, directly from the signal, as in claim 2) that has utilised information on the change in level of the metabolism indicator to provide an output that actually enables the user to assess (i.e. realise or know) that enrichment is taking place. The Examiner has suggested that changing from a manual process into an electronic process as in claim 2 is obvious. However this is not so. For a start, the type of analysis conducted must be one that can be completed without human involvement. That is not possible for tests that involve human analysis of FTIR graphs, change in colour or similar. The present application shows data proving that a signal representing a terminal electron acceptor level can be used to calculate change in the level of that molecule over time, and through this shows the actual enrichment of the microorganism as it takes place.

We provide the following comments in response to the specific comments made by the Examiner.

In the first two paragraphs in Supplemental Box 1, the Examiner has asserted that each individual step mentioned in claim 1 has been used in the art, and as a consequence the combination is not inventive, even though the Examiner has provided no information proving that the specific combination claimed *would* have been made. By comparison, in patents for new mechanical devices, individual mechanical components making up a new device are usually known, but unless there was a motivation or awareness of how those components can be put together to produce a device that achieves something new, then the device is considered to be inventive.

In the present situation, the method involves a combination of components and processing steps, performed on a test substrate, that produces a new result – the identification of a microorganism that can metabolise the test substrate, within a convenient and quick system, without excessive external manipulation, that can be repeated again and again for different test substrates using the same methodology. The claimed method can be applied to test substrates regardless of what that test substrate is. This is achieved because a deliberate decision has been made to use a signal of a probe for a terminal electron acceptor (a molecule involved in respiration), and the this signal is used to assess the change in level of that molecule involved

in respiration over the time frame of the enrichment, to produce an output (electronically directly, according to claim 2) that actually represents metabolism of the test substrate, without the operator having to measure the amount of test substrate in the vessel over time. Any time the prior art uses an off-line process, and a different type of metabolism indicator, shows that the prior art was teaching away from the claimed invention. The Examiner has provided no indication that any party had any motivation at all to create a method having this broad applicability, that uses each of the claimed steps in the specific combination claimed. No prior art documents discuss the desire to have a method that has broad applicability irrespective of the test substrate used – without this motivation, there is no background that would lead the person skilled in the art to consider making changes to any process shown in the prior art so as to arrive at the presently claimed invention. It is the combination of features that enable broad application irrespective of the test substrate, that provide the inventive step.

The Examiner states "online monitoring of various parameters of microbial metabolism or growth is not new..." This is correct when considered on its own, however, microbial metabolism is not, on its own, an indicator of enrichment. For example, a microbe may be metabolizing a substrate and be in stationary phase (i.e. dead/not growing) as is exemplified in the following reference ("introduction to Biocatalysis using enzymes and micro-organisms, Cambridge University Press, 1995, pp49). To clarify that the present process utilises a technique that is shown in the examples to accurately reflect enrichment, which process relies on change in the level of the terminal electron acceptor, we have amended claim 1. This makes claim 1 more clearly different from processes where simple dissolved oxygen levels are measured, without then assessing the change in the level of that molecule (as in OUR), which must be used to establish that enrichment is occurring.

The Examiner states, "...neither enrichment cultures nor the use of continuous flow chemostats/bioreactors is new". This is correct but the claims define more than this, and neither of these types of prior art devices can be used on their own, without the additional features we have specified in the claims, for carrying out the enrichment procedure of the type claimed. Chemostats and bioreactors can and have been used to carry out enrichment of microbial populations. Our invention combines the use of a chemostat (or bioreactor) with the on-line measurement of the change in level of a terminal electron acceptor (such as OUR(Oxygen Uptake Rate), rather than just DO), a combination that has not been used for microbial enrichment or selection.

The Examiner states that "it is common practice to determine parameters other than disappearance of substrate, for example, production of metabolites or biomass, or measurements of respiration such as ATP formation, are commonly used parameters". Whilst these procedures can be used to monitor an enrichment process, the stated procedures cannot be used to differentiate between cells that may be in stationary phase (i.e. not growing) or dead and in the case of production of biomass, does not necessarily correlate to enrichment (could be acclimation). This is why the claimed use of a real-time indicator of living cells – being the terminal electron acceptor consumption, such as oxygen consumption. The claimed process

specifically uses the rate (i.e. change in claim 1) of oxygen consumption as a measure of microbial activity.

In arguing that claim 1 lacks novelty over D5 and D1, the Examiner deems the term "acclimatisation" to be synonymous with the term "enrichment". The two terms are not synonymous and there is no evidence in the Examiner's citations to consider them synonymous. For example, in reference D5 the term "enrichment" is never used by the authors because there is no indication that the microbial population has changed in its microbial profile (i.e. the genera/species of microbes). The D1 citation only uses the term "enrichment" to describe the increasing concentration of chemicals not microbes and prefers to use the term "acclimation" correctly, to describe the adaptation of the biological consortium to TPE, PCE, CT and HCB.

It is our position, as supported by the general way in which the term is used in the specification, and in the art, that enrichment is where one microbial genus/species/ is selected over another, whereas acclimation is the adaptation of the same microbial strain to a different set of conditions. The former involves one microbe "dying off" or not growing and another surviving the condition, whilst the latter can occur simply by switching genes on and off as required by the same microbe. As further evidence that the two terms are not synonyms, we present two example publications that use the two terms as independent terms and not as synonyms. (J. Agric. Food Chem, 2000, vol 48 pp4341-4351 and Can. J. Microbiol. 1999, vol 45 pp520-9). The first paper describes the "enrichment" of a microbial consortium from sewage followed by "acclimation" of the enriched consortium to increasing levels of a pollutant. They obtained improved biodegradability of their polluting chemical by the enriched culture only after acclimating the already enriched microbes. These authors make a clear distinction between the two terms throughout their manuscript. The second paper makes the same clear distinction. Accordingly, the evidence shows that enrichment is different to, and does not encompass acclimation. Thus, prior art process involving acclimation are not relevant to the novelty of the present claims.

Regarding D5, the Examiner states that "Measurements were taken of COD, free cyanide, thiocyanate, copper, zinc, and iron concentrations and of MLVSS. Of these, COD and MLVSS are indicators of microbial metabolism. It is apparent that oxygen is required to metabolize cyanide and thiocyanates." Measurements may well have been taken (although it is not described how DO was measured) but they were definitely NOT used for control of their acclimation process. That is, D5 does not disclose step (d) in claim 1, which requires that the change in level of the metabolism indicator be used to assess selective enrichment of the microorganism. Furthermore, measurements were carried out by removal of samples and NOT through a probe that takes a reading in the vessel (i.e. not online). MLVSS is not a terminal electron acceptor, as now required by claim 1, and in any event is not a true metabolism indicator, since the compounds making up this material could include a range of complexes with the inorganic precipitates, and therefore will be contaminated and not correctly reflect the biomass.

Although these points do not necessarily go to novelty of claim 1 over D5, we would also like to highlight the following:

- These authors used dissolved oxygen to maintain a bioremediation process and not to control it for enrichment/discovery of microbes.
- The schematic picture of the "bioreactor" does indicate a dissolved oxygen probe. Thus, the information in the document points to the DO being measured in a sample taken out of the vessel.
- The authors have not drawn any parallels between dissolved oxygen levels and removal of pollutants. Specifically, authors did not use DO as an assay for the monitoring of pollutants (they assayed pollutants off-line and separately). This is supported by the absence of any such graphs or tables. The authors only studied the impact of DO on the removal of pollutants (pg 344 M&M's). This does not equate to the requirement in claim 1 that an "output" is provided "showing the change in level of the metabolism indicator which is based on the signal of the probe to enable assessment of selective enrichment..." Further, with respect to claim 2, there is no output produced *electronically* that shows this.
- There is no indication that the biomass actually metabolised the pollutants. Pollutant removal may well have been through bioadsorption (i.e. sticking on the microbes) or volatization (see pg 345 Results and Discussion).
- D5 only mentions the removal of inorganic substrates and not organic substrates. D5 does not therefore teach the preferred feature set out in claim 20, which is particular to organic substrates.

Regarding D1, as stated above, that process involves acclimation of a microorganism, rather than enrichment. Evidence has been submitted to demonstrate that enrichment does not encompass acclimation.

With regard to D1, the Examiner also states that "IR spectroscopy is used in the citation to measure chlorinated substrates, the technique can readily be used to monitor the majority of organic molecules." This statement is not correct for a typical solution that would be used in the claimed process. For example, IR spectroscopy has considerable problems differentiating mixtures of organic compounds such as would be found in a fluid containing a mixed microbial population, such as the preferred inoculum of sewage or activated sludge and the test substrate. In fact FTIR spectroscopy has found little use in monitoring complex mixtures of compounds, as would typically be present in the fluid medium claimed. Measurement of changes in the level of the terminal electron acceptor, such as oxygen consumption, as used in the claimed invention, can be applied to a broader range of substrates (and complex mixtures) without the above mentioned interferences and can be done in real time. We also reiterate the comments made in our response to the first Written Opinion.

For D1 the Examiner also states that " ....it would be obvious to one skilled in the art that the methods of the citation could be used to monitor enrichment of dechlorinating microbial cultures, as it provides a way to measure rate of substrate disappearance, which is clearly a function of successful enrichment". The rate of substrate disappearance is not a clear function

of enrichment as was pointed out by D5 where bioadsorption or volatization could be the causes of substrate disappearance rather than any type of enrichment. This is why monitoring consumption of the terminal electron acceptor (through monitoring the level of this over time) is the best way of detecting enrichment since it is a direct measure of LIVING cells which in turn can be used as a direct measure of enrichment.

We understand the basis for the objections raised by the Examiner to date, and through the clarifying amendments proposed, and the additional information presented above, we believe that the novelty and inventiveness of the claimed method has been shown. The claimed invention needs to be read in the context of its capability of discovering microorganisms capable of metabolising vastly differing substrates, all using the same technique, and therefore the same equipment set-up. From the above, it can be seen that the prior art does not disclose each feature claimed, and does not foreshadow such a process having the advantage of repeatability on a broad range of test substrates.

Favorable reconsideration is respectfully requested.

Yours faithfully
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IN THE MATTER OF International Patent Application No. PCT/AU2004/001577 in the name of COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION entitled METHOD FOR MICROBE AND ENZYME DISCOVERY Our Ref: JSB:SP:FP20705

SECOND STATEMENT OF PROPOSED AMENDMENTS

2.	Page 63	Cancel this page and substitute therefor replacement page 63 lodged herewith

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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method for selectively enriching for a microorganism able to metabolise a test substrate, and/or the enrichment of an enzyme involved in the metabolism of the test substrate, the method comprising the steps of

a) providing a population of microorganisms in a vessel,

b) feeding fluid into the vessel at a controlled flow rate commencing with an initial flow rate, the fluid comprising a nutrient medium and, for at least part of the feed period, the test substrate,

c) producing a signal indicative of the level of a metabolism indicator which is a terminal electron acceptor, over the time-frame of the enrichment, wherein the signal is produced from a probe that takes a reading in the vessel, and

d) providing an output showing the change in level of the metabolism indicator which is based on the signal of the probe to enable assessment of selective enrichment of a microorganism that metabolises the test substrate, and/or the enrichment of an enzyme produced by the microorganism that is involved in the metabolism of the test substrate.

2. The method of claim 1, wherein the output is produced electronically directly from the signal, such that the output is provided on-line.

3. The method of claim 1 or claim 2, wherein the method further comprises presetting conditions to be met by the signal output to result in a change in the fluid flow rate, and changing the flow rate at which fluid is fed into the vessel when the conditions are met, wherein the preset conditions are a combination of a predetermined period of time and a preset value range within which the signal must remain for the predetermined period of time.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method for selectively enriching for a microorganism able to metabolise a test substrate, and/or the enrichment of an enzyme involved in the metabolism of the test substrate, the method comprising the steps of

a) providing a population of microorganisms in a vessel,

b) feeding fluid into the vessel at a controlled flow rate commencing with an initial flow rate, the fluid comprising a nutrient medium and, for at least part of the feed period, the test substrate,

c) producing a signal indicative of the level of a metabolism indicator which is a terminal electron acceptor, ~~represents cellular activity other than direct measurement of the change in the level of the test substrate~~ over the time-frame of the enrichment, ⊗ and

d) providing an output showing the change in level of the metabolism indicator which is based on the signal to enable assessment of selective enrichment of a microorganism that metabolises the test substrate, and/or the enrichment of an enzyme produced by the microorganism that is involved in the metabolism of the test substrate.

2. The method of claim 1, wherein the output is produced electronically directly from the signal, such that the output is provided on-line.

3. The method of claim 1 or claim 2, wherein the method further comprises presetting conditions to be met by the signal output to result in a change in the fluid flow rate, and changing the flow rate at which fluid is fed into the vessel when the conditions are met, wherein the preset conditions are a combination of a predetermined period of time and a preset value range within which the signal must remain for the predetermined period of time.

⊗ wherein the signal is produced from a probe that

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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method for selectively enriching for a microorganism able to metabolise a test substrate, and/or the enrichment of an enzyme involved in the metabolism of the test substrate, the method comprising the steps of

a) providing a population of microorganisms in a vessel,

b) feeding fluid into the vessel at a controlled flow rate commencing with an initial flow rate, the fluid comprising a nutrient medium and, for at least part of the feed period, the test substrate,

c) producing a signal indicative of the level of a metabolism indicator which represents cellular activity other than direct measurement of the change in the level of the test substrate over the time-frame of the enrichment, and

d) providing an output based on the signal to enable assessment of selective enrichment of a microorganism that metabolises the test substrate, and/or the enrichment of an enzyme produced by the microorganism that is involved in the metabolism of the test substrate.

2. The method of claim 1, wherein the output is produced electronically directly from the signal, such that the output is provided on-line.

3. The method of claim 1 or claim 2, wherein the method further comprises presetting conditions to be met by the signal output to result in a change in the fluid flow rate, and changing the flow rate at which fluid is fed into the vessel when the conditions are met, wherein the preset conditions are a combination of a predetermined period of time and a preset value range within which the signal must remain for the predetermined period of time.